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Physiological modulation of the vitality of Scots pine trees by atmospheric ammonia deposition

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8. General discussion

8.1. Introduction

Exposure of Scots pine to gaseous NH_3 affects the metabolism at various levels. At the cellular level, assimilation of NH_3 causes metabolic changes, resulting in an increase of the N concentration of the shoots and excess release of H^+ to the cytoplasm. At the physiological level, NH_3 increases net photosynthesis, stimulates shoot growth, and changes biomass partitioning between shoots and roots. The effects of NH_3 are modified by other gaseous pollutants, SO_2 and O_3, and elevated CO_2. The various responses of photosynthesis and shoot growth suggest that the N status of the tree is very important in determining the effect of NH_3, beneficial or detrimental. The results of the research presented in this thesis are discussed and the chapter ends with a critical review on the criteria used by policy makers to arrive at a reduction of atmospheric N deposition, including suggestions on the use of physiological parameters in air pollution research.

8.2. Modification of metabolism by NH_3 exposure

Effect on glutamine synthetase and free amino acid pool

Gaseous NH_3 significantly increased GS activity in one-year-old needles (Chapters 3, 4, 5 and 6; see also Table 8.1). Ammonia counteracted the decrease in GS activity caused by exposure of the trees to SO_2 and O_3 (Chapters 4 and 6). The increase in GS activity was accompanied by an increase in concentration of N-containing metabolites as soluble proteins, free amino acids and leaf pigments (Chapter 3).

The N derived from gaseous NH_3 was mainly incorporated into arginine, glutamic acid, aspartic acid and glutamine (Chapter 3). These amino acids, together with proline and histidine, formed the major part of the free amino acid pool in needles of trees in a forest stand (Chapter 7), exposed to relatively high atmospheric NH_3 concentrations (20-30 μg m^{-3} yearly-average and maximum concentration above 400 μg m^{-3} 1 hour-averaged; Van Elzakker *et al.*, 1994). Clearly, changes in GS activity can be used as an indicator for foliar uptake and assimilation of gaseous NH_3. Glutamine, glutamate and aspartate occupy a central position in the pathway for amino acid biosynthesis (Rowland *et al.*, 1988). Arginine is a readily available source of N because it has the highest N/C ratio among amino acids. It is also a precursor of polyamines, which are important metabolites in resistance to air pollutants (Rowland-Bamford *et al.*, 1989). Obviously, the fact that needles of Scots pine readily incorporate gaseous NH_3 into amino acids, may be advantageous

when the trees are simultaneously subjected to other stress conditions.

Effect on glutamate dehydrogenase

The increase in GDH activity of the needles upon NH_3 exposure (Chapter 7) was related to carbohydrate limitation in the shoots, rather than to direct effects of gaseous NH_3 on GDH activity. The increase in GDH activity after exposure to SO_2 and O_3 was also associated with an increased demand for carbohydrates (Chapters 4 and 6). These results agree with a deaminating role of GDH, which catalyses the oxidation of glutamate when 2-oxoacids are deficient. GDH, thus, provides the tricarboxylic acid cycle with 2-oxoglutarate (Robinson *et al.*, 1992).

Effect on N concentration

Exposure of Scots pine trees to NH_3 resulted in a significant increase in N concentration of current-year needles (formed the present year), but not in one-year-old needles (formed the previous year; Chapter 5). The latter have a low stomatal conductance and, thus, a low N uptake. Also, N translocation from one-year-old to current-year needles takes place during the growing season (Millard & Proe, 1993). This effect of needle age agrees with results by Van der Eerden & Pérez-Soba (1992) and Van der Eerden *et al.* (1992). The effect of NH_3 on N concentration of one-year-old needles was variable, ranging from a significant increase (Chapters 2, 3 and 4) to insignificant changes (Chapter 5). Such variable effects were probably caused by a different rate of N translocation from one-year-old to current-year needles. In fumigated trees fertilized with $(NH_4)_2SO_4$, the N concentration in needles increased with NH_3 concentration, while in unfertilized trees the needle N concentration hardly differed between NH_3 fumigation treatments (Chapter 2). In unfertilized trees, the N concentration increased non-linearly with NH_3 concentration (Chapters 2, 3 and 4), in agreement with observations on *Lolium perenne* exposed for five days to NH_3 concentrations ranging from 327 to 1266 $\mu g\ m^{-3}$ (Wollenweber & Raven, 1993). In conclusion, foliar N concentration is a very insensitive indicator of NH_3 exposure and assimilation.

One year NH_4^+ fertilization did not affect the N concentration in current-year needles during the first four months (August to December; Chapter 2); evidently, N was not taken up by the roots or was allocated to other tree parts. During the same period, fumigation with NH_3 significantly increased N concentration in current-year needles, which may prolong growth and retard hardening, thus, enhancing susceptibility of trees to frost (Huttunen & Laine, 1983; Dueck *et al.*, 1991).

Effect on nutrient balance

It has been said that nutrient ratios are better indicators of needle nutrient status than the individual nutrient concentrations (Van den Burg, 1985). Gaseous NH_3 decreased K/N and P/N ratios by an increase in the N concentration (Chapters 2 and 4), as observed in *Pseudotsuga menziesii* saplings fumigated with NH_3 (Van der Eerden *et al.*, 1992), and in *P.*

sylvestris forests close to intensive livestock farms (Van Dijk & Roelofs, 1988; Ferm *et al.*, 1990). Exposure to SO_2 induced a decrease in foliar P and K concentrations (Chapter 4), indicating that the simultaneous occurrence of NH_3 and SO_2 in forest ecosystems might cause a serious nutrient imbalance in the needles, as showed by K/N and P/N ratios.

8.3. Modification of shoot-root interactions by NH_3 fumigation

Needle NH_3 uptake versus root NH_4^+ uptake

Foliar NH_3 uptake mainly depends on stomatal conductance and is regulated by factors such as light intensity and air humidity (Van Hove *et al.*, 1987, 1989). Under controlled-environment conditions, absorption of NH_3 into the leaf is by diffusion (Van Hove *et al.*, 1987), provided that gaseous NH_3 concentrations are above the "compensation point" which is less than 5 $\mu g\ m^{-3}$ (Farquhar *et al.*, 1980; Duyzer *et al.*, 1994). Needles absorb NH_3, even when trees are well-supplied with N from the soil (Chapter 2). Consequently, gaseous NH_3 exposure leads to an increase of the N pool in the shoots, and more N becomes available for transport to other tree parts with a high N demand, *e.g.* roots during fast growth (Nambiar & Fife, 1987).

Large amounts of translocated N might reduce the uptake of NH_4^+ by the root itself as well as translocation from root to the shoot by an effect on xylem loading. In this way, uptake of NH_3 by the needles may reduce uptake of NH_4^+ by the roots. Allocation of NH_3-derived N from shoots to roots has been shown after $^{15}NH_3$ fumigation of grasses (Dueck *et al.*, 1991) and maize (Grundmann *et al.*, 1993). The shoot-root N signal could be an organic (amino acids) and/or an inorganic N compound (NO_3^-, NH_4^+). The amino acids, cycling in the plant, might inhibit net uptake of NO_3^- in the roots (Clarkson, 1986; Muller & Touraine, 1992). Exposure of trees to gaseous NH_3 resulted in an increase in the amino acid pool concentration, more noticeable arginine (Chapter 3). By arginine cycling, a high level of this amino acid in the roots seems possible, which in turn may strongly inhibit NO_3^- uptake, as observed in *Arabidopsis thaliana* (Doddema & Otten, 1979) and in *Glycine max* (Muller & Touraine, 1992). Further experiments are needed to test the hypothesis that arginine plays an important role as signal compound in the cycling-pool of amino-N, additional to its role as N-storage in conifers (Durzan, 1968; Pietiläinen & Lähdesmäki, 1986) and as an indicator of nutrient imbalance by excess NH_4^+ (Rabe & Lovatt, 1986; Näsholm & Ericsson, 1990; Chapter 7).

Needle growth versus root growth

Gaseous NH_3 stimulates photosynthesis rate in conifers, which has been associated with an increase in nitrogen and chlorophyll content of the needles and with an increased stomatal conductance (Van Hove *et al.*, 1992; Van der Eerden & Pérez-Soba, 1992). Also, shoot biomass is enhanced (Van der Eerden & Pérez-

Soba, 1992), which results in an increase in the shoot/root ratio.

Exposure to gaseous NH_3 induced a secondary effect on roots: the percentage of mycorrhizal tips respect to total root tips and the mycorrhizal number in the total root system were reduced (Chapter 6), probably due to an NH_3 modification in the quantity and quality of root exudates, rather than by a reduced allocation of carbohydrates to roots as suggested by Van der Eerden *et al.* (1992). A further decrease in the mycorrhizal number in the total root system, together with a trend to increase in the biomass of fine roots was observed when NH_3 was applied at an elevated CO_2 level; apparently, the extra carbon supply to the shoots via elevated CO_2 was not invested in mycorrhizal symbiosis but in growth of fine roots. The latter may counteract the reduction in nutrient uptake due to decreased mycorrhizal infection (Marschner & Dell, 1994).

Gaseous NH_3 may temporarily delay an O_3 induced decrease in net CO_2 assimilation rate, by a transient increase (Van Hove & Bossen, 1994); the latter may result in a higher C allocation to roots and thus fulfil the C demands of mycorrhizas (Chapter 6).

8.4. Toxicity of NH_3 pollution depends on the N status of the tree

Once gaseous NH_3 has entered the leaf, it is dissolved in the water film of the mesophyll cell walls to form NH_4^+. Ammonium may accumulate and cause visible toxic effects, if the assimilation capacity of the needles is exceeded (Fangmeier *et al.*, 1994). The assimilation of NH_3 itself may also lead to toxicity through an increase in N compounds and the release of H^+ in the cytoplasm (Raven, 1988). Damage will occur when the pH buffering capacity of the cytoplasm is exceeded (Pearson & Stewart, 1993).

No visible injury was observed after exposing conifer trees to gaseous NH_3 at concentrations of 40 to 260 $\mu g\ m^{-3}$ (Chapters 2, 3, 4, 5 and 6; Dueck *et al.*, 1991; Van der Eerden *et al.*, 1992; Van der Eerden & Pérez-Soba, 1992; Van Hove & Bossen, 1994). The majority of the previously mentioned experiments resulted in an increase in organic N compounds, an increase in growth of current-year needles, and an increased net CO_2 assimilation. On the other hand, two experiments showed no stimulation of needle growth or net CO_2 assimilation by NH_3 (Chapter 6; Dueck, pers. commun.). We suggest that differences in response to gaseous NH_3 depend on the N status of the needles prior to the fumigation. The following arguments support this hypothesis:

(1) if the N concentration is lower than optimal for growth, NH_3-derived N may alleviate N deficiency; net CO_2 assimilation will increase, since photosynthesis rate will increase when the limiting N concentration increases above the minimum N concentration of the needles (Brix, 1981), resulting in extra needle biomass production ("fertilization");

(2) if the N concentration is supraoptimal, more N may be reallocated from the needles to other tree parts with a large N demand, like fine roots, or storage tissue. The time sequence during seasonal growth will determine whether the amount of extra N is used for internal cycling or for storage (Millard & Proe, 1992, 1993). Net CO_2 assimilation will not be further stimulated and needle growth will not be enhanced ("saturation");

(3) after a long term exposure of conifers to gaseous NH_3, less carbohydrates will be available for NH_4^+ assimilation, resulting in NH_4^+ accumulation and leading to visual injury ("toxicity").

Extending the hypothesis of the dependence of NH_3 toxicity on the N status of the tree to the field situation, conifer trees in the neighbourhood of intensive stock breeding farms will show visible symptoms depending on the physiological and growth conditions that relate to their N status:

(1) if the needle N concentration is lower than 1.8-2%, which is considered a level optimal for growth (Van den Burg, 1990), trees will not show visual symptoms of toxicity nor physiological or growth disturbances: "the green trees" (Van Dijk & Roelofs, 1988);

(2) if the needle N concentration is around the optimal range, visual symptoms will also not be observed, but trees will show metabolical and physiological alterations, indicating N excess, like high arginine concentrations and low K/N and P/N ratios: "the control trees" in Chapter 7;

(3) if the needle N concentration is far above the optimal range (2.3-3.3%), trees will show serious nutrient imbalance, growth disturbance and yellowing of the needles (Van Dijk & Roelofs, 1988; Kaupenjohann et al., 1989). Ferm et al. (1990) concluded that in Scots pine trees close to fur animal farms, the observed tree damage and growth disturbances were positively correlated with the high total N and NH_4^+ concentrations of the needles.

8.5. Use of physiological parameters in air pollution research

Current policy measures to reduce the deposition of atmospheric NH_3 on forest ecosystems are based on the critical loads (CLOs) for N, expressed as the amount of N deposited in the soil (kg N ha^{-1} y^{-1}) and aim for long-term protection of ecosystems (10-100 years). The CLOs for N are mainly based on the results of fertilization experiments in which N is supplied to the soil. In this way several aspects which are important for forest ecosystems are neglected. Firstly, in reality there is shoot N uptake as well, which differs in physiological consequences. Foliar uptake of gaseous NH_3 considerably contributes to the N content of the needles, and may reduce root NH_4^+ uptake as well as translocation from root to shoot (Chapter 2). In addition, foliar NH_3 uptake can disrupt the structure of the epicuticular wax layer (Van der Eerden et al., 1992)

and can acidify the cytoplasm of the mesophyll cells (Wollenweber & Raven, 1993). Consequently, short-term responses, shoot-root relations and direct disturbance of foliar NH_3 uptake are overlooked in CLOs data.

Secondly, the proportion of the different N forms (NH_4^+, NO_3^-) and their different physiological importance for trees should be taken into account: roots of Scots pine seedlings may accumulate more NH_4^+ in the presence of NO_3^- without toxic effects (Vollbrecht & Kasemir, 1992). In the same way, leaves are more susceptible to NH_4^+ when they do not assimilate NO_3^-. Nitrate reduction generates 0.78 mol OH^- per mol N assimilated, which counteracts the acidification caused by the production of cytoplasmic protons as result of NH_4^+ assimilation (Raven, 1988; Pearson & Stewart, 1993; Wollenweber & Raven, 1993).

In the third place, the two fluxes of NH_3-N within the system tree-air (uptake from the air and emission from the tree) and their physiological effects are omitted, since CLOs are based on the effects of the net flux of N. Additionally, differences in susceptibility between and within tree species to atmospheric NH_3 (Dueck *et al.*, 1992b; Smits, 1992) should be included in CLOs.

The use of physiological parameters may help to estimate the health of forest ecosystems in a sensitive way. Differences in susceptibility of tree species to atmospheric NH_3 may be assessed as differences in GS activity in the needles, which indicate a difference in capacity to assimilate high concentrations of atmospheric NH_3 (Chapter 7). In the same way, differences in buffering capacity index (BCI) between tree species occur (Pylypec & Redmann, 1984), which reflects differences in capacity to maintain cytoplasmic pH (Pylypec & Redmann, 1984; Pearson & Stewart, 1993). The rate of photosynthesis per unit leaf N, viz. photosynthetic nitrogen use efficiency (PNUE), could be a useful indicator to assess the ability of trees to tolerate N excess. Above a minimum N concentration in the leaf, PNUE will increase up to a maximum and then decline again (Lambers et al., 1989). The relationship between PNUE and needle N concentration for a tree species, may provide an estimation of the "fertilizing", "saturating" and "toxic" effects of atmospheric NH_3, as described in the previous section.

In conclusion, the combined use of physiological parameters will provide a useful approach to estimate and sustain health of forest ecosystems with respect to total N deposition.